

# James A. Baker Institute for Animal Health Annual Report, 1989

College of  
Veterinary Medicine  
Volume 39



CORNELL  
UNIVERSITY



*This report honors  
those whose  
generosity sustains  
the Institute's  
independence and  
commitment to  
excellence and the  
pursuit of truth.*





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*Although  
Baker Institute  
investigations  
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Douglas McGregor



## A Message from the Director

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Nineteen eighty-nine was a year in which we made gratifying progress on two fronts. First, our research produced the rewarding new findings described in this report; second, we completed a major facilities improvement program. The expansion, though costly and temporarily disruptive, has positioned the Baker Institute for success in meeting the challenges all research institutions will face in the 1990s. Those challenges include the competition for research resources and skilled personnel and the increasing sophistication and cost of biomedical research.

Institute research is sponsored by federal and state governments, foundations, and industry, as well as by the many clubs, associations, and individuals listed in this report. The United States Public Health Service is the largest single sponsor, providing approximately half of the Institute's operating income. During the past several years government funding has increased modestly, but not at a rate sufficient to compensate for inflation or for the actual costs of research. Moreover, a larger portion of federal funds has been directed to clinical trials and targeted initiatives such as research on AIDS. All of the subjects given priority are important and need to be addressed; however, the shift in emphasis has left proportionally less for research intended to benefit animals. In 1989 the Institute experienced a modest decline in federal awards. The decline was less than 5 percent below last year's record level; nevertheless it presages a trend that poses a major challenge for the Institute.

We have been greatly encouraged that awards and gifts received from other sources compensated to some degree for the decline in government sponsorship of Institute research. As we look to the future, it is evident that we will be obliged to rely increasingly on the generosity of individuals, foundations, and other groups who recognize the importance of research in maintaining the health and quality of life of companion animals.

Although Baker Institute investigations principally benefit dogs and horses, there is growing awareness that these studies also

affect the lives of other species, including our own. Colin Parrish's research on parvoviruses is a notable case in point. That work began ten years ago when Leland Carmichael and Max Appel first isolated canine parvovirus and identified it as the cause of a serious disease of dogs. Colin has carried those investigations forward in a new direction, using modern methods of molecular biology. By determining fine genetic and structural differences between closely related parvoviruses of dogs, cats, and wildlife species, Colin is adding to our knowledge of how viruses evolve in nature. His findings will shed light on the process by which new viruses occasionally emerge to infect previously resistant species.

The work described by Colin and by others in this report makes use of equipment that is both expensive to acquire and costly to maintain. Other costs have increased as well. They have been driven partly by inflation and partly by restrictions governing the use of radioactive materials, infectious agents, and toxic substances that are unavoidable by-products of our efforts. Such materials must be handled in ways that ensure the health and safety of our personnel and the quality of the environment. The Institute is committed to meeting the most rigorous compliance standards in that regard.

Another issue that we must address concerns the use of animals as research subjects. As specified in its charter, the Institute "operates for the purposes of controlling animal diseases and the teaching of all that pertains to those diseases." The hopes and aspirations of our founders have been richly rewarded in the development of vaccines now used by veterinarians to prevent infectious diseases of dogs and other species and in the development of methods for detecting and treating such diseases. That work continues. Because the safety of vaccines and the efficacy of diagnostics and treatment strategies can be evaluated only by testing them in the species for whose benefit they are intended, we must continue to use animals in such research in order to protect the population at large. We limit the number tested to the very minimum

*We have an  
up-to-date, newly  
expanded research  
facility, a capable  
and dedicated staff  
of men and women,  
a distinguished  
advisory council,  
and a growing  
number of friends  
and benefactors.*



Susan Hamlin

required to obtain useful information, and stringent precautions are taken to ensure their safety and care. It should also be remembered that the Institute has pioneered the development of alternative methods to the use of animals as research subjects. Notable in that connection was the establishment of the first laboratory for the study of animal viruses in cell cultures. Elsewhere in this report, Dr. Nancy Burton-Wurster describes the use of cultured cartilage fragments to determine how cartilage degenerates in the joints of animals with osteoarthritis. Other investigators are using cultured cells to produce antibodies in order to ascertain how the immune system is engaged by viruses, bacteria, and parasites.

At the annual meeting of the advisory council, Jacqueline Lindsay was honored with the 1989 Arthur F. North, Jr. Canine Service Award. Mrs. Lindsay was chosen in recognition of her outstanding commitment to canine health as evidenced in her establishment within the Milwaukee Foundation of the Steven S. Lindsay Memorial Fund. Mrs. Lindsay created the fund to honor and perpetuate her late son Steven's affection for people and animals, and the Institute has benefited substantially as a result of her actions. We gratefully acknowledge her generous assistance.

The past year also marked the passing of a great friend and benefactress of the Institute, Frances Rowles Van Brunt. Some of our friends knew Mrs. Van Brunt through her active involvement with the Irish Wolfhound Club of America. For her dedication to furthering the goals of the Institute, she was named the 1986 recipient of the North award. We note her loss with profound regret.

The significant progress made in recent years would not have been realized without the help and assistance of the distinguished scientists and community leaders who make up our advisory council. Its function is to advise the director about all aspects of the Institute's operations and to assist in its long-range planning. This year Dorothy R. Donnelley retired as chairwoman of the council. Under Dorothy's leadership the

Institute has grown and been transformed into the modern, well-equipped laboratory complex that it is today. Words cannot convey to her our gratitude for her generosity, interest, and encouragement, and we are enormously pleased that she will continue as a member of the council. The position of chairman has been assumed by William Rockefeller, who has been a member of the advisory council since 1979. His connections to the Institute extend much further back in time; Bill's father and aunt helped found this institution. We are confident that under his leadership the Institute will continue to maintain its preeminent position in veterinary medical research. That confidence is firmly based in the knowledge that we have an unrivaled combination of assets—an up-to-date, newly expanded research facility, a capable and dedicated staff of men and women, a distinguished advisory council, and a growing number of friends and benefactors. No institution could wish for more.



Douglas D. McGregor



Jeanne Truelsen



## Staff of the Baker Institute

### *Administration*

**Douglas D. McGregor**, director: B.A., M.D., U. of Western Ontario; D. Phil., Oxford U.  
**Susan Howell Hamlin**, administrative manager: B.S., Elmira College  
**Nancy D. Combs**, accounts coordinator  
**Carlene M. Furch**, development assistant  
**Anita S. Hesser**, secretary to the director  
**Florence C. Huth**, administrative aide  
**Sharon E. Morrow**, accounts assistant  
**Letha B. Padgett**, administrative aide  
**Jeanne G. Truelsen**, assistant director for development: B.A., M.A., Miami U.  
**Robert D. Wakeman**, office assistant

### *Emeritus*

**Ben E. Sheffy**, Caspary Professor of Nutrition, Emeritus: B.S., M.S., Ph.D., U. of Wisconsin

### *Laboratories*

#### **Giralda Laboratory for Canine Infectious Diseases**

**Leland E. Carmichael**, John M. Olin Professor of Virology: A.B., D.V.M., U. of California; Ph.D., Cornell  
**Maria Fernandez-Maillo**, graduate research assistant: D.V.M., U. Complutense, Madrid  
**Jean C. Joubert**, research support specialist  
**Sandra J. Bond**, laboratory technician: B.S., U. of Guelph

#### **Hadley C. Stephenson Laboratory for the Study of Canine Diseases**

**Max J. G. Appel**, professor of virology: Dr. med. vet., U. of Hannover; Ph.D., Cornell  
**Mary Beth Matychak**, research technician: U. of Evansville  
**Susan E. Pearce-Kelling**, laboratory technician: B.S., M.S., Cornell

#### **John M. Olin Laboratory for the Study of Canine Bone and Joint Diseases**

**George Lust**, professor of physiological chemistry: B.S., U. of Massachusetts; Ph.D., Cornell  
**Nancy Burton-Wurster**, senior research associate: B.A., M.S., Ph.D., New York U.  
**Rory J. Todhunter**, graduate research assistant: B.V.Sc., U. of Sydney; M.S., Michigan State U.; Dip. ACVS, Cornell  
**Juergen Steinmeyer**, postdoctoral fellow: Ph.D., U. of Bonn  
**Margaret S. Peery**, laboratory technician: B.S., Ohio State U.  
**Raymond O. Recchia**, laboratory technician: B.S., SUNY College at Fredonia  
**Alma J. Williams**, laboratory technician: B.A., U. of Pennsylvania; M.S., Cornell; AALAS accreditation  
**Gerald J. Pijanowski**, visiting associate professor: D.V.M., Cornell; M.S., Ph.D., Purdue U.

#### **Immunology Laboratory**

**Robin G. Bell**, associate professor of immunology: B.Sc., Australian National U.; Ph.D., John Curtin School of Medical Research  
**Ching-Hua Wang**, research associate: M.D., Peking Medical School; Ph.D. Cornell  
**Karen M. Trotter**, postdoctoral associate: B.S., M.S., Ph.D., Cornell  
**Ali Ahmad**, graduate research assistant: D.V.M., M.Sc., West Pakistan Agricultural University (Faisalabad, Pakistan)  
**Theodore Llana III**, graduate research assistant: B.S., U. of Maryland  
**Anthony T. Vella**, graduate research assistant: B.A., SUNY College at Buffalo; M.S., Rensselaer Polytechnic Institute  
**Lincoln S. Adams**, research technician: B.S., Hobart College; AALAS accreditation  
**Hui-Qing Bai**, visiting associate professor: M.D., Da Lian Medical College  
**Xiu-Feng Lü**, visiting associate professor: M.D., Jin Zhou Medical College

#### **Mucosal Immunity Laboratory**

**Judith A. Appleton**, assistant professor of immunology: B.S., Indiana U.; M.S., Ph.D., U. of Georgia

Melissa S. Carlisle, graduate research assistant: B.V.Sc., U. of Queensland  
Laura K. Hanson, graduate research assistant: B.S., U. of Washington  
Lucille F. Gagliardo, laboratory technician: B.S., Southhampton College  
Lynn A. Usack, laboratory technician: B.S., Cornell  
Tie-Yan Han, visiting fellow: B.M., Capital Medical College, Beijing

### **Equine Genetics Center**

Douglas F. Antczak, associate professor of immunology: B.A., Cornell; V.M.D., U. of Pennsylvania; Ph.D., Cambridge U.  
John A. Angelos, graduate research assistant: B.S., Cornell  
William L. Donaldson, graduate research assistant: B.V.Sc., U. of Queensland  
Juli K. Maher, graduate research assistant: B.S., D.V.M., U. of Illinois  
Chong-Hui Zhang, graduate research assistant: M.S., Nanjing Agricultural U.  
Debra A. Dougherty, research support specialist: B.S., U. of Rhode Island  
Sherry L. Dickens, laboratory technician: B.S., Cornell  
Cheryl M. Rigas, laboratory technician: B.S., Cornell

### **Donnelley Laboratory of Immunochemistry**

Douglas D. McGregor, professor of immunology: B.A., M.D., D. Phil.  
Alan D. King, graduate research assistant: B.S., U. of Alabama; D.V.M., Auburn U.  
Mark D. Hulsebosch, laboratory technician: B.S., St. Joseph's U.

### **Bostwick Laboratory of Molecular Biology**

Colin R. Parrish, assistant professor of virology; B.Sc.; Massey U.; Ph.D., Cornell  
Shwu-Fen Chang, graduate research assistant: B.S., Taipei Medical College; M.S., National Taiwan U.  
Mary Lisa Strassheim, laboratory technician: B.S., Cornell

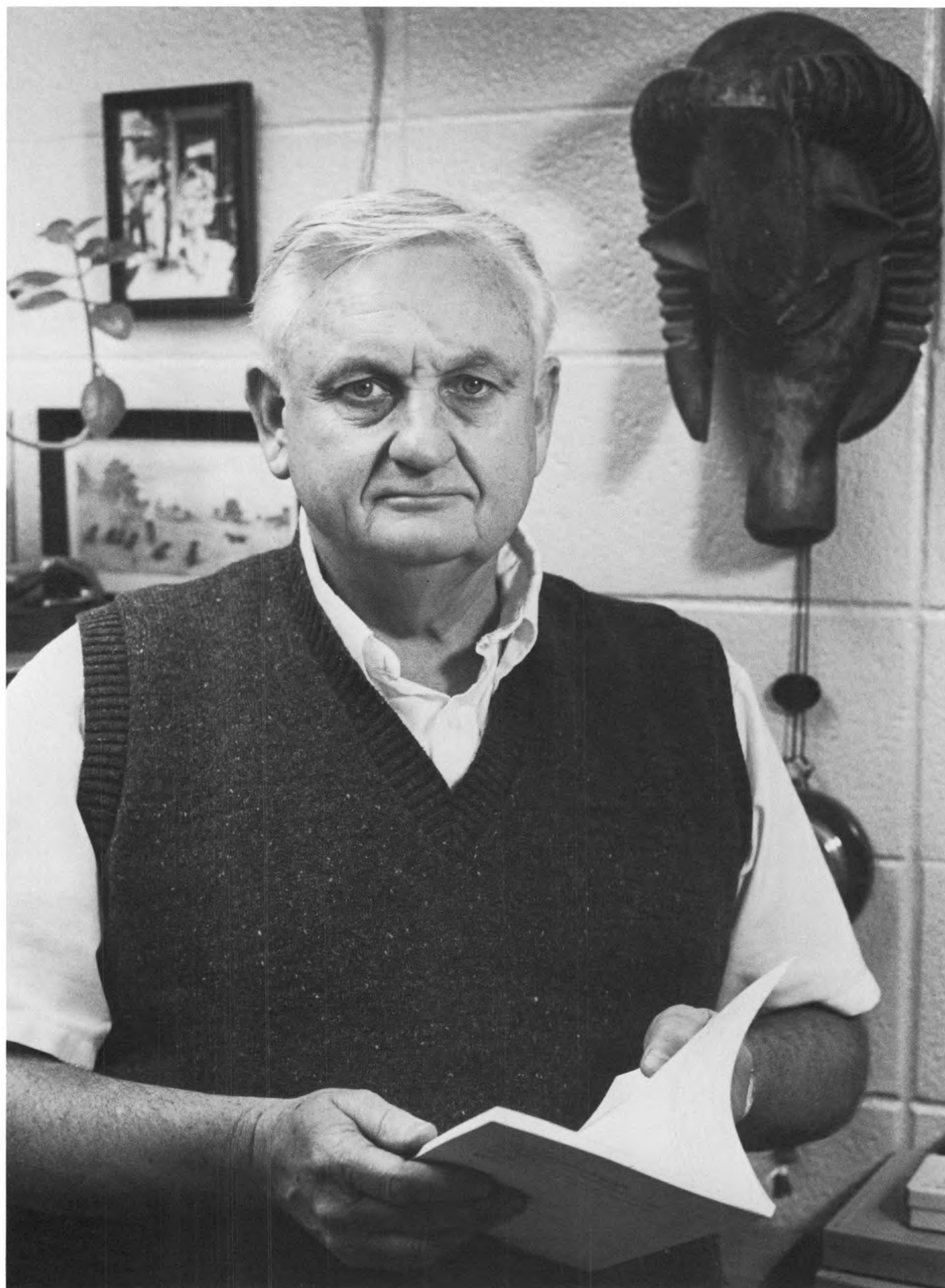
### **Research Service Laboratories**

Thomas M. Bartol, Jr., research support specialist (flow cytometry): B.S., Cornell  
Paul J. Lutwak, research support specialist (chromatography): B.A., B.S., Miami U.  
Jeannette R. Carney, laboratory attendant  
Roy L. Barriere, vivarium supervisor: AALAS accreditation  
Jack H. Arcangeli, Jr., animal technician  
Rita H. Bamforth, animal technician  
Raymond M. Combs, animal technician: AALAS accreditation  
Raymond J. Corey, animal technician: A.A.S., SUNY Agricultural and Technical College, Delhi; AALAS accreditation  
Kevin T. Draiss, animal technician: A.A.S., SUNY Agricultural and Technical College, Delhi; B.S., Cornell, AALAS accreditation  
James C. Hardy, research support specialist: B.S., Cornell; AALAS accreditation  
Mark Meal, animal technician  
Laura A. Michel, animal technician: A.A.S., SUNY Agricultural and Technical College, Farmingdale; B.S., Cornell; AALAS accreditation  
Anastasia E. Newell, animal technician: AALAS accreditation  
Arthur D. Howser, maintenance supervisor, buildings and grounds  
Kathleen L. Crispell, custodian  
Ralph L. Crispell, custodian  
Richard E. Daniels, maintenance mechanic  
Robert A. Foux, maintenance mechanic  
Russell F. Haus, building coordinator

*Glassware  
Animal Care*

*Maintenance*

*We are evaluating two  
diagnostic texts for  
canine brucellosis  
that could spare dogs  
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serological reports.*



Leland Carmichael



## Giralda Laboratory for Canine Infectious Diseases

### *Canine Brucellosis*

We have continued to evaluate two diagnostic tests for canine brucellosis developed in our laboratory. One procedure, an improved screening test, uses a mutant *B. canis* strain; the other is an immunodiffusion test in agar gel that employs cytoplasmic protein antigens of the organism. The screening test has an accuracy of approximately 90 percent—a significant improvement over the slide and tube agglutination tests presently used for laboratory confirmation of canine brucellosis. The agar gel diffusion test is technically more difficult to perform than the screening test. Although it is unsuitable for routine office use, it is more reliable than the screening test and superior to other serologic methods developed so far for the diagnosis of brucellosis. Its practical utility lies in the evaluation of sera that yield indeterminate results with other methods or where false positive results are suspected.

This year we evaluated 272 serum samples from ninety-seven kennels in states that span the country. The majority were problem cases in which an owner or veterinarian was baffled by results reported by a diagnostic laboratory. More than 90 percent of the cases we examined had previously been judged positive by slide or tube agglutination methods. Using the new agar gel diffusion test, we concluded that 74

percent of these dogs did not have brucellosis. Seventy of the samples were seropositive. Most were from dogs in which the diagnosis of brucellosis was established by blood culture. The positive group included German Shepherds, a Doberman, Pomeranians, Pointers, Fox Hounds, and, in largest number, Beagles.

The foregoing results validate the new tests and suggest that their general use would spare many valuable dogs that would otherwise be killed because of false positive or "suspicious" serological reports.

### *Minute Virus of Canines*

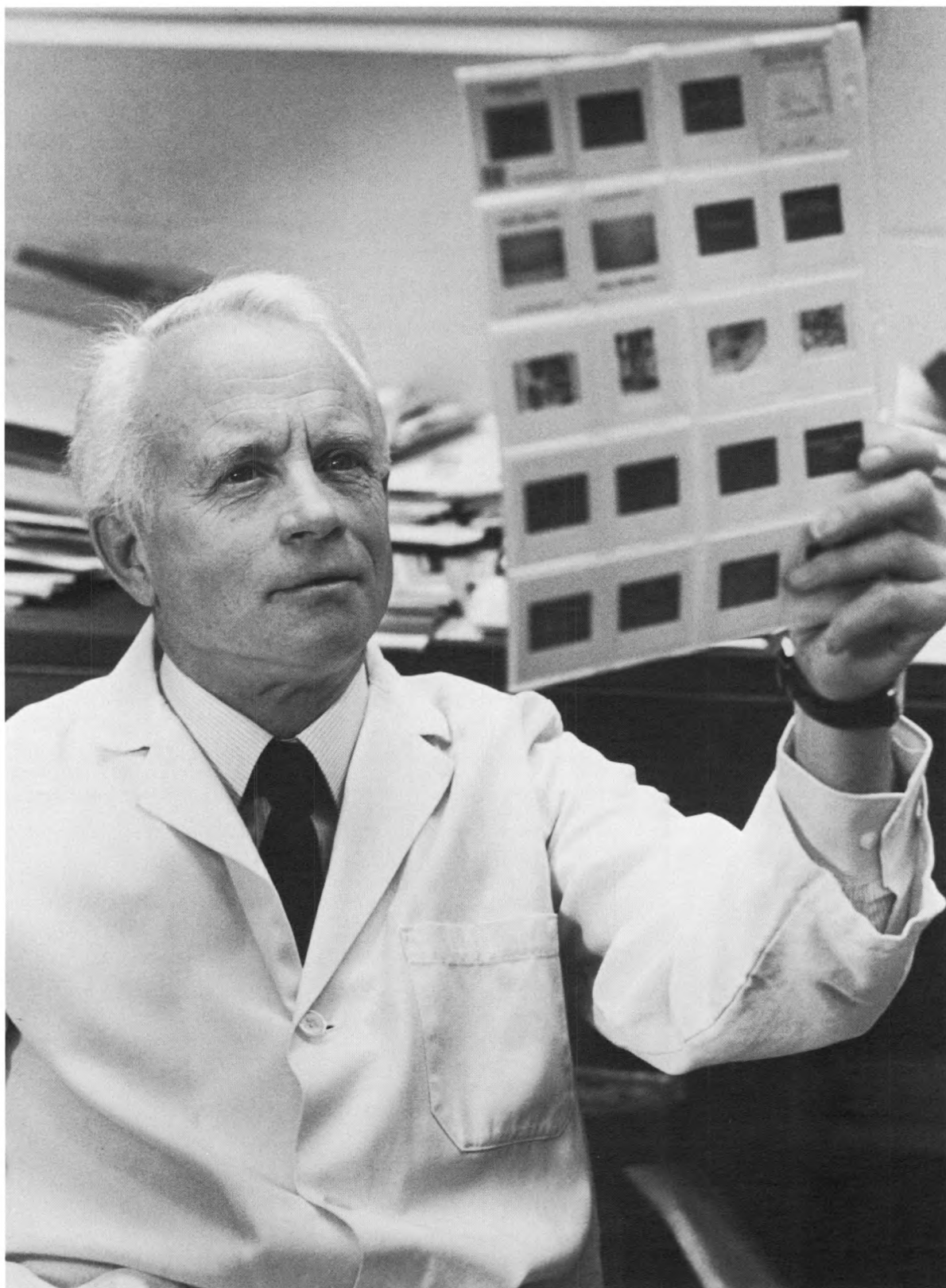
Our research this year also focused on the capacity of minute virus of canines (MVC, also known as canine parvovirus type 1) to cause disease in the developing canine fetus. Field observations have suggested the involvement of MVC in fetal resorptions as well as in enteritis and death of pups less than two weeks old. We were fortunate in this project to have the collaboration of Dr. Akira Hashimoto, on leave from Gifu University in Japan. Dr. Hashimoto is an expert in infectious diseases of the canine reproductive tract. Together we established experimentally that MVC can cause fetal infections after oral-nasal or intravenous inoculations. Intra-amniotic inoculation of the virus also caused lethal infections in fetuses after an incubation period of approximately fifteen days. Inoculated fetuses, and sometimes adjacent fetuses, were infected; those more distantly located in the uterus were spared. It will be necessary to develop more-sensitive assays for MVC before we can ascertain the capacity of the virus to cause disease under natural conditions. The long incubation period and the time between fetal death and parturition may limit the use of conventional methods (viral isolation, immunofluorescence) for viral diagnosis and assessment of the disease-causing potential of this virus.

*Leland E. Carmichael*



Sandra Bond

*Individual strains of  
canine distemper  
virus (CDV) vary in  
their capacity to cause  
brain disease in  
unvaccinated dogs.*



Max Appel

## Hadley C. Stephenson Laboratory for the Study of Canine Diseases

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This laboratory has long been interested in canine distemper virus (CDV) from the standpoint of both vaccine development and the capacity of CDV to cause brain disease in dogs. This year we tested a larger number of virulent CDV isolates and found them to be indistinguishable by serological tests. The results imply that currently available CDV vaccines are adequate and there is no need to develop "special" vaccines to prevent distemper caused by certain strains. Nevertheless, we have determined that individual strains of CDV vary in their capacity to cause brain disease in unvaccinated dogs. Some strains cause an acute disease that involves mainly neurons and the grey matter of the central nervous system. Dogs infected by such strains either die or recover rapidly. Other strains induce a delayed or chronic form of the disease, with involvement of glial cells (predominantly astrocytes) and the white matter. Demyelination, the loss of nerve sheath in the brain, is a common sequela. This form of CDV-induced encephalitis resembles the lesions seen in multiple sclerosis, a human disease of unknown etiology but one that is believed to be caused by an infectious agent.

In collaboration with Sue Pearce-Kelling and Dr. Brian Summers we have tested the hypothesis that different brain cell types are infected by individual strains of CDV that differ according to the patterns of disease described above. Dissociated brain cell cultures were prepared from newborn pups. The cultures consisted predominantly of astrocytes, but neurons, oligodendroglial cells (the cells responsible for myelin, or nerve sheath, production), and microglial cells (macrophages of the brain) also were present. These brain cell cultures were infected with either a CDV strain that induces acute disease in dogs or with a strain that induces chronic disease.

We have a panel of antibodies with which we can identify the different types of cells. Using these reagents, we demonstrated that oligodendroglial cells are only sparingly infected by either strain of CDV. Thus it appears unlikely that oligodendroglial cells are killed by virus, leading to demyelination.

Likewise, neurons were rarely infected by CDV, regardless of the pattern of disease. The difference between acute (neuronal) and chronic (demyelinating) encephalitis therefore cannot be explained by a tropism, or affinity, of CDV for neurons or oligodendrocytes. Microglial cells were readily infected by both strains of CDV, however. That finding suggests that microglial cells are responsible for the spread of CDV in the brain. We noted a marked difference in the infection of astrocytes by different strains of CDV. A CDV strain that induces acute disease infected virtually all astrocytes within one week. By comparison, another CDV strain that induces chronic disease required five to six weeks to cause a similarly widespread infection. The results correlate with the time course of the infection in dogs.

We also observed rapid infection of astrocytes in brain cultures infected by a CDV vaccine strain that is widely used around the world to immunize dogs against distemper. The virus replicates under normal conditions only in lymphatic tissues. In rare circumstances, however, the vaccine virus reaches the brain, causing a fatal infection, so-called postvaccinal distemper encephalitis. The ability of the virus to infect astrocytes may explain the rapid tempo of this uncommon but dramatic phenomenon.

A variety of specimens obtained from dogs were screened for the presence of "new" viruses or other infectious agents. One finding of interest was the isolation of LaCrosse virus from a dog with encephalitis (submitted by Dr. Lenn Harrison and serotyped by Dr. Robert Shope of New Haven, Connecticut). The virus is transmitted by insects and is a public health concern. Infection with LaCrosse virus is fairly common in the human population, but its isolation from a dog has not previously been reported.

*Max J. G. Appel*



*We are monitoring  
the relationship  
between laxity and  
the development of  
hip dysplasia under  
rigorously controlled  
conditions.*



George Lust

## John M. Olin Laboratory for the Study of Canine Bone and Joint Diseases

### *Hip Dysplasia*

A major objective of our research is to ascertain the role of joint laxity in the development of dysplastic hips. Laxity is the opposite of tightness. In the context of hip dysplasia, it refers to the degree of motility of the head of the thigh bone within the acetabulum, or joint socket, of the pelvic bone. Conventional wisdom holds that laxity favors the development of hip dysplasia. We are monitoring this relationship under circumstances in which other factors that may contribute to the development of hip dysplasia, such as nutrition and exercise, are rigorously controlled. We will continue our observations throughout the lifetime of the subject dogs or until they develop clinical or radiological evidence of hip dysplasia. Gerald Pijanowski is collaborating with us in this project while on sabbatical leave from the College of Veterinary Medicine at the University of Illinois.

The foregoing studies may be successful in their goal of establishing the relationship between joint laxity and the development of hip dysplasia, but they will not reveal how or why some joints exhibit a greater degree of laxity than others. One possibility is that the degree of laxity may be influenced by factors affecting the growth of bones in the region of the hip joint. We are testing this hypothesis in experiments conducted jointly with Dr. Robert Kaderly, an orthopedic surgeon in the Department of Clinical Sciences at Cornell's College of Veterinary Medicine. Dr. Kaderly has performed a surgical operation on Beagle pups that accelerates the rate at which the acetabulum is formed. In each of two animals, one joint was surgically manipulated, and the other was not. In both dogs the operated joints developed X-ray evidence of hip dysplasia within six months, whereas the unoperated joints were free of disease. These results give credence to the view that premature development of the hip joint favors the development of hip dysplasia. However, one must be cautious in drawing conclusions of that sort from experiments performed on only two dogs. Therefore it is our intention to further evaluate the relationship

between acetabular development, joint laxity, and the development of hip dysplasia, using not only Beagles but also Labrador Retrievers, which are more susceptible to the disease.

*George Lust*

### *Osteoarthritis*

#### **PATHOGENESIS:**

Articular cartilage is by necessity a resilient tissue, being subject as it is to frequent and intense mechanical forces caused by weight bearing and movement of the joint. A healthy hip joint, for example, can support loads of up to five times body weight. Because mechanical factors are as much a part of the environment of cartilage as are biochemical factors, it is reasonable to expect that the chondrocyte, the cell responsible for maintaining the integrity of the cartilage matrix, will respond to mechanical stress.

In last year's annual report we described a project initiated jointly by Juergen Steinmeyer, a postdoctoral fellow in our laboratory, and Peter Torzilli, a biomechanical engineer at the Cornell Medical College. Dr. Torzilli has designed an instrument that enables us to apply controlled static and intermittent compressive forces up to ten times body weight to articular cartilage explants. The initial challenge was to determine whether cartilage could be successfully cultured and maintained in the environment of this machine. After that was established, Dr. Steinmeyer began to explore the metabolic response of cartilage to applied pressure. Two aspects of cartilage metabolism were evaluated: (1) the synthesis and accommodation of fibronectin, and (2) the loss of proteoglycan, a major structural element of the cartilage matrix. Measurements were made in a uniform piece of cartilage that was subjected to a predetermined load applied at a standard frequency. The findings were of great interest. Under the conditions of mechanical loading Dr. Steinmeyer determined that fibronectin synthesis in cartilage explants was reduced threefold from that in similar

*Understanding the function of fibronectin in normal cartilage would help to explain how the accumulation of fibronectin in diseased tissue might disrupt normal metabolic processes.*



Nancy Burton-Wurster



explants not subjected to mechanical stress. Moreover, loading resulted in a reduction in the amount of proteoglycan released into the culture medium. In those two respects, the "loaded cartilage" behaved like cartilage in a normal joint.

The foregoing results are preliminary and need to be confirmed; nevertheless, they suggest that cartilage requires certain mechanical signals to function normally. Those findings are just the beginning, however, because additional questions must be asked and answered. For example, was the application of the load in a cyclic manner important to the results, or would a static load give the same results? What will happen when the frequency and magnitude of the load are changed? And what will happen when some important biochemical mediators of cartilage metabolism are added in conjunction with mechanical stress? Our long-term goal is to learn how to alter cartilage metabolism in vitro to mimic what we observe in tissue from osteoarthritic joints. That capability would contribute significantly to understanding the pathogenesis of the disease.

Another key to the pathogenesis of osteoarthritis lies in explaining the function of fibronectin in normal cartilage. Such an understanding would allow us to postulate how the accumulation of fibronectin in diseased tissue might disrupt normal metabolic processes. Our observations so far suggest that there is a tight interaction between a cartilage proteoglycan and a portion of the cartilage fibronectin. Most proteoglycans exist in the cartilage in the form of very large aggregates formed by the association of a great many of the proteoglycan molecules with one molecule of hyaluronic acid. It is those aggregates that contribute to the organization and biomechanical properties of the cartilage. Plasma fibronectin cannot bind to hyaluronic acid, but cartilage fibronectin, when associated with proteoglycan, can. It then can be found associated with the large proteoglycan aggregates. This finding implies that cartilage fibronectin may have a role in cartilage organization or disorganization, and we will be

examining our results carefully in the coming year to better understand their implications.

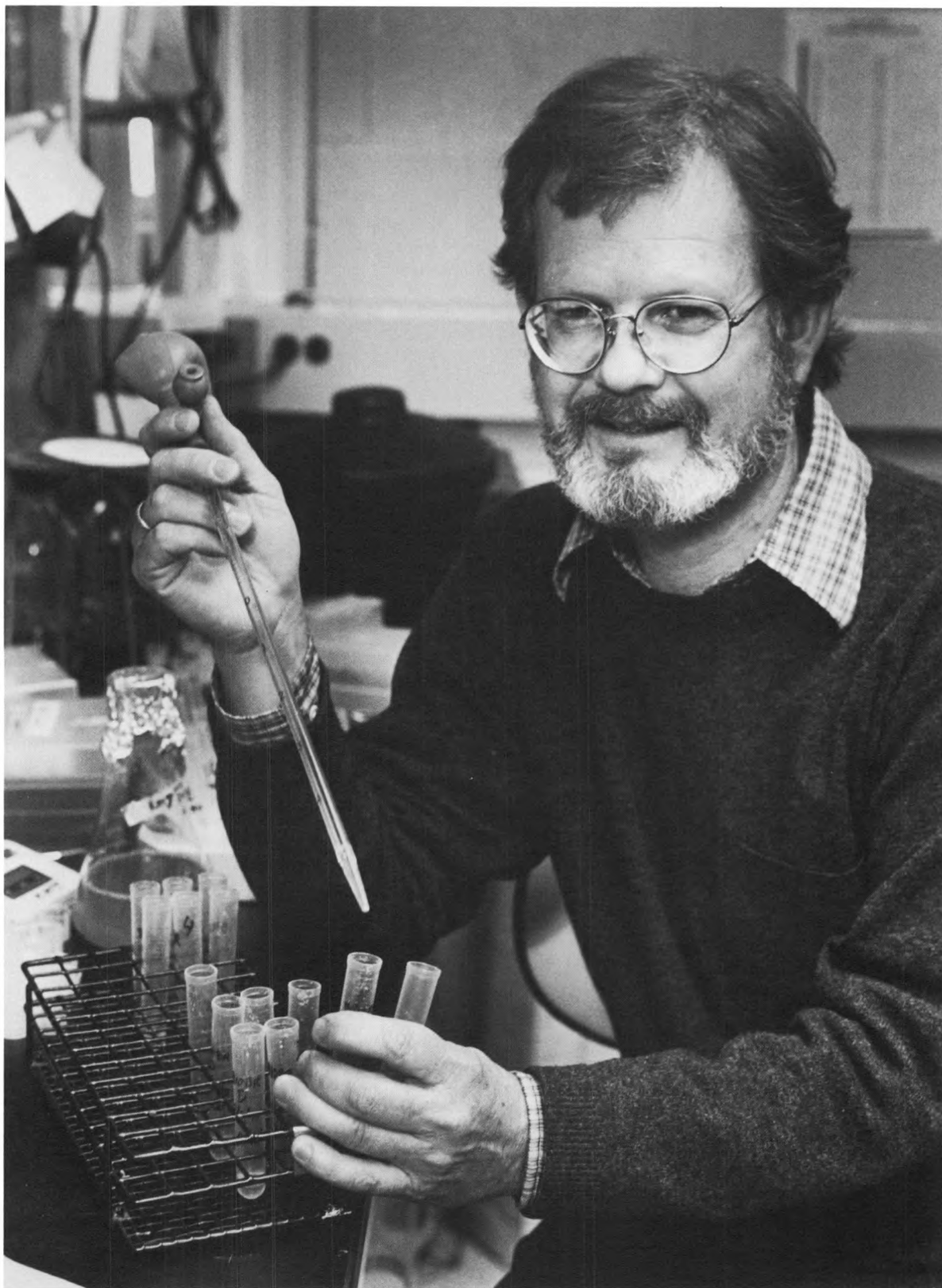
#### DIAGNOSIS:

Several laboratories have made attempts to develop a blood test for osteoarthritis that would enable them to detect the disease early and follow its progression. In all cases the rationale is that molecules normally found only in the articular cartilage are released into the blood stream when cartilage degenerates. Although several cartilage-specific molecules have been studied, no effective diagnostic test has yet been developed.

Because fibronectin increases so dramatically in osteoarthritis, we have reasoned that some cartilage fibronectin may be found in the blood. Detection of cartilage fibronectin in the blood, however, will be impossible unless we can make a monoclonal antibody that recognizes only cartilage fibronectin in the presence of large amounts of plasma fibronectin. This has proven to be very difficult, however, because the occurrence of a hybridoma producing a cartilage-specific antifibronectin antibody is expected to be rare and therefore difficult to select by conventional screening methods. Rory Todhunter, a veterinarian who is a Ph.D. student in our laboratory, and Margaret Peery, a technician, are attempting to use an innovative technique, suggested by Alan King of this Institute, that involves labeling the hybridomas with fluorescent-labeled plasma or cartilage fibronectins and then using the fluorescence-activated cell sorter (FACS) to select those hybridomas that recognize only cartilage fibronectin-specific epitopes separately from those that recognize epitopes common to both plasma and cartilage fibronectin. Preliminary experiments suggest that Dr. King's technique has promise; if so, it will be valuable not only to us but also to anyone faced with a similar selection problem.

*Nancy Burton-Wurster*

*Our evidence suggests that rapid expulsion is a two-stage process, first involving immune cells in the intestinal wall and then involving antibodies.*



Robin Bell

Several years ago we began studies of an intestinal immune reaction called "rapid expulsion." As the name implies, the key feature of rapid expulsion is the speed with which parasites—in our case *Trichinella spiralis*—are rejected from the intestine. Rapid expulsion occurs when an animal previously infected with *T. spiralis* ingests a meal containing infectious *T. spiralis* larvae. Studies in this laboratory and in others have demonstrated that a kinetically similar reaction occurs in dogs, guinea pigs, sheep, cattle, and mice infected with other parasites. Those findings suggest that rapid expulsion is a reaction of demonstrable significance to antiparasite immunity in the gut. Despite the importance of rapid expulsion as an acquired defense, knowledge about its underlying mechanism has yielded grudgingly to experimental study. For the past three years a visiting veterinarian from Pakistan, Ali Ahmad, has been investigating the phenomenon in rats. Earlier this year Dr. Ahmad was awarded a Ph.D. degree for his research, and his results are summarized in this report.

Dr. Ahmad's investigations employ the experimental techniques of "adoptive" and "passive" transfer, in which cells or serum from an animal immunized as the result of a *T. spiralis* infection are transferred to an uninfected recipient. At a predetermined interval after the transfer the recipient is infected with *T. spiralis* larvae and examined for its capacity to express rapid expulsion. The examination involves the enumeration of intestinal parasites twenty-four hours after the infection and comparison of the results with the parasite burden in an animal given neither cells nor serum from an immune donor. In fact, groups of rats are used in studies of this sort in order to ascertain the consistency and statistical significance of the results. A reduction in the number of worms in recipients of immune cells, immune serum, or both is an indication that rapid expulsion has occurred.

Dr. Ahmad quickly demonstrated that both cells and serum from an immune donor are required for a normal rat to rapidly expel

*T. spiralis*. The cells he used were obtained from the thoracic duct lymph of donor rats, thereby establishing the identity of the cells as lymphocytes. In subsequent experiments Dr. Ahmad demonstrated that the active cells were members of a particular subset of lymphocytes known as T-helper cells. Further analysis revealed that the helper cells were identical to those that Dr. Ching-Hua Wang from this laboratory had demonstrated to be the mediators of immunity against a later stage in the parasite's development, the adult *T. spiralis* worm.

In a series of rather complicated experiments, Dr. Ahmad demonstrated that immune cells and serum act sequentially to affect the rapid expulsion of *T. spiralis*. The evidence suggests that the phenomenon is a two-stage process, the first involving immune cells located in the intestinal wall and the second involving antibodies. A possible explanation of how cells and antibodies cooperate in the expulsion of *T. spiralis* emerged from yet another series of experiments. By separating the various classes of serum antibodies into their individual constituents, Dr. Ahmad demonstrated that the antibody responsible for rapid expulsion was Immunoglobulin E. IgE is known for its capacity to bind to mast cells, which are present in large numbers in intestinal tissue. In other circumstances the binding of IgE to mast cells is critical to the expression of allergic reactions such as those associated with hay fever or food allergies. Therefore it is plausible that the binding of IgE to intestinal mast cells is the critical event needed to trigger an allergic reaction that is hostile to the parasite, promoting its expulsion from intestinal epithelium. The results provide the first unequivocal demonstration of the involvement of IgE in an intestinal antihelminth reaction that is protective for the host.

Other laboratory personnel involved in the project were Lincoln Adams and Dr. Wang.

Robin G. Bell

*Antibody to  
T. spiralis, which  
causes trichinosis, can  
be chemically cleaved  
and still retain its  
ability to passively  
immunize rat pups.*



Judith Appleton



### ***Maternal Immunity to Trichinella spiralis***

*Trichinella spiralis*, the causal agent of trichinosis, can provoke an immune response in adult rats. That immunity can be transferred from an infected donor to her suckling pups. Last year we reported that this maternally transmitted immunity is conveyed by antibody, but only some subclasses of Immunoglobulin G (IgG) antibody can passively immunize infant rats. This year we substantiated and extended those findings by showing that the parasite provides a potent stimulus for the production of some antibody subclasses whereas others are produced in far smaller amounts.

As part of her Ph.D. dissertation research, Melissa Carlisle demonstrated that antibody to *T. spiralis* can be chemically cleaved and still retain its ability to passively immunize rat pups. Cleavage destroys portions of the molecule that confer on antibodies subclass-specific properties, such as the ability of the antibody to bind to certain cells or to complement. (Complement is in fact a collection of proteins whose sequential activation is central to the expression of a wide variety of immune responses.) Although chemical cleavage of the antibody molecule impedes its interaction with cells or complement, it does not impair its capacity to bind to antigen. Thus, the ability of truncated antibodies to immunize infant rats against *T. spiralis* indicates that the event critical to the creation of immunity occurs in the binding of antibody to its target antigen rather than in any of the functions peculiar to the subclasses that contain protective antibodies.

Last year we mentioned that the antibodies that confer protection on infant rats bind to the same antigen molecules. Those molecules exist in several forms and are present both on the surface of the parasite and in its secretion products. A major objective of our research is to identify and purify antigens only to which protective antibodies bind. If we are successful in that endeavor, we can then determine the

amino acid sequence of the molecules and, subsequently, the genes that control the expression of the subject antigens.

### ***Immunity against Equine Influenza Virus***

Our research on equine influenza continues to focus on interactions between the causal virus (EIV) and antibodies that can neutralize the virus. Laura Hanson, a graduate student in this laboratory, has developed a virus neutralization assay that may be helpful in identifying previously unknown neutralization sites on EIV.

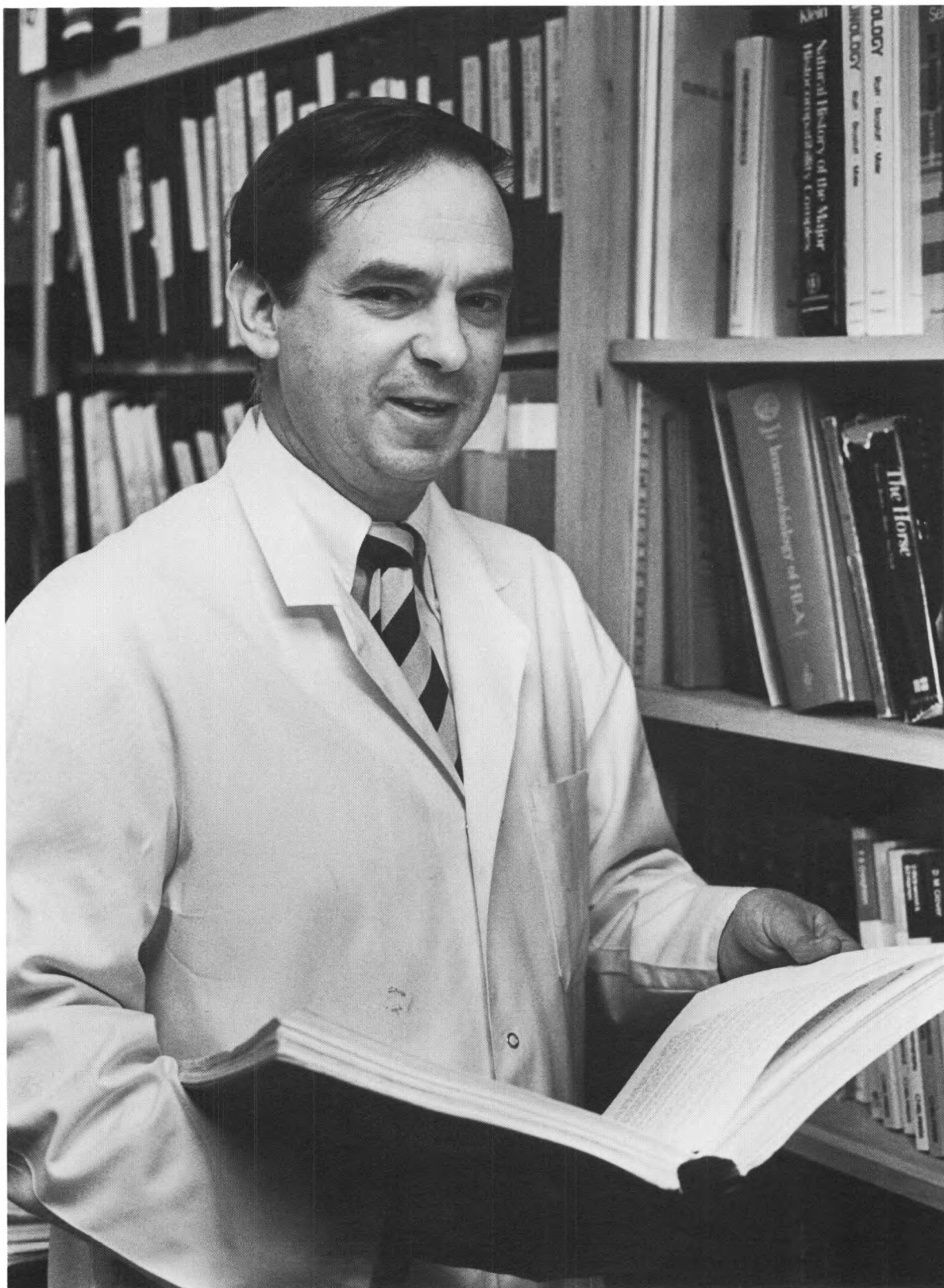
On another front, we continued our studies of the immune response to EIV in horses. Last year we reported that EIV does not change as rapidly as human influenza viruses do. This year we determined that horses make antibodies against surface molecule of EIV in much the same fashion that people do.

*Judith A. Appleton*



Melissa Carlisle

*Can we apply what  
we have learned  
about immunological  
regulation in the  
placenta to other  
situations, such as  
clinical organ  
transplantation and  
tumor immunology?*



Douglas Antczak

### *Gene Expression in the Placenta*

The placenta, in particular its outer cell surface, the trophoblast, is the barrier that both separates and joins mother and fetus during pregnancy. That interface is an extraordinarily complex one. It functions as a channel for the transport of nutrients from mother to fetus and the removal of fetal waste products to the maternal circulation for clearance and elimination. A number of elegant selective mechanisms operate to permit those transfers.

The placenta also serves an important immunological function. It is well known from work in many species that even early in pregnancy the fetus is antigenically mature in that it expresses histocompatibility molecules coded by genes inherited from both parents. In most pregnancies, however, the fetus is protected from destruction by the maternal immune system, even when the mother has become immunized against fetal histocompatibility antigens.

Perhaps the most important immunological question concerning the fetal-maternal relationship is whether major histocompatibility complex (MHC) antigens are expressed by trophoblast cells. That question is still debated by reproductive biologists, but most accept the hypothesis that trophoblast cells surrounding the placenta suppress genes coding for MHC antigens and thereby present a poor antigenic target to the immune system. In most species, maternal immune responses to fetal MHC antigens either do not occur or are recorded only late in pregnancy. In the horse, however, maternal immune responses occur in virtually all pregnancies. Therefore, the question of expression of MHC antigens by the trophoblast takes on special significance in this species.

Our laboratory has studied many aspects of those responses during the past ten years. We have learned that the specialized trophoblast cells of the chorionic girdle express high levels of fetal MHC antigens for a brief period between days thirty and forty-five of pregnancy. Those cells are probably the source of the fetal MHC antigens, which stimulate the maternal immune response. Interestingly, the

descendant endometrial cup trophoblast cells turn off the genes that control the expression of MHC antigens. That action may be essential to the ability of the cup cells, which can live for up to three months burrowed deep within the maternal tissues of the uterus, to secrete the chorionic gonadotropin hormone thought to be critically important to the success of pregnancy in the horse. Much of this work was performed by Bill Donaldson as part of his dissertation research.

A number of important questions remain. What mechanisms operate in trophoblast cells to control the expression of MHC antigens? Can we apply what we have learned about immunological regulation in the placenta to other situations, such as clinical organ transplantation and tumor immunology? Juli Maher, who joined our laboratory in July, is addressing the first of those questions.

### *Equine Tumor Virology*

Sarcoid is the most common tumor of horses and a clinically important problem for many horses and horsemen. Our studies of this condition over the past five years have confirmed the links between horse breed and prevalence of the tumor and between the MHC type and susceptibility. John Angelos, a student in the combined D.V.M.-Ph.D. program at Cornell, has been conducting research on the problem as part of a joint project involving Dr. Carmichael's laboratory and our own. Mr. Angelos has demonstrated papilloma virus DNA in most sarcoid tumors but not in normal tissues from infected horses or in skin from normal horses.

The complex relationships among host, virus, environment, and method of treatment will be explored in the spring of 1990 at an international workshop on equine sarcoid sponsored by the Dorothy Russell Havemeyer Foundation. Experts in virology and genetics, as well as veterinary pathologists and clinicians, will meet to discuss recent progress and plan a prospective study of sarcoid that might answer some of the outstanding questions about the condition.



*Our research has  
provided valuable  
material for studies of  
the immune system  
of the horse.*



Debra Dougherty



### ***Equine Leukemias and Immunodeficiencies***

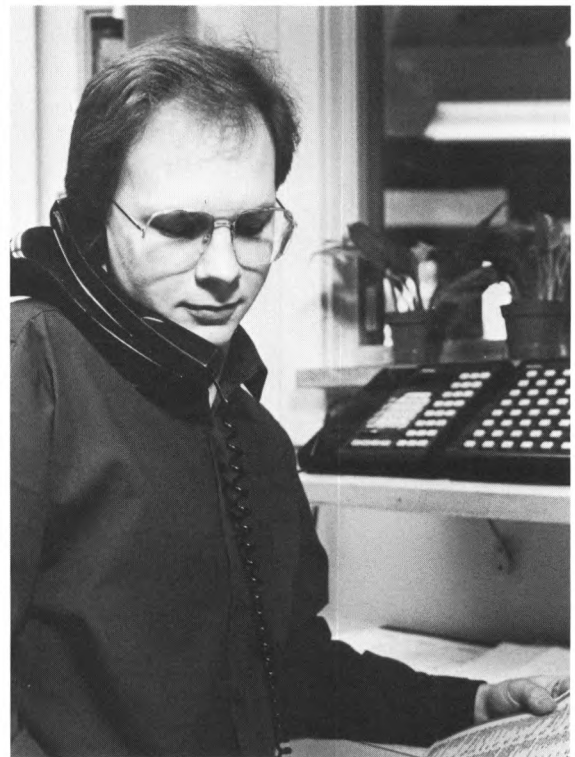
During the past three years the work of Chong-Hui Zhang, a graduate student in this laboratory, has produced a panel of monoclonal antibodies as markers for lymphocyte subpopulations in the horse. The antibodies were generated primarily to characterize the composition and function of the maternal lymphocytes that accumulate around and appear to destroy the endometrial cups in early horse pregnancy. However, the antibodies also can be used to characterize equine lymphocyte populations in many other situations.

This year we had the opportunity to work with large-animal clinicians from Cornell and the University of Pennsylvania on several cases of leukemia and immunodeficiency in horses. Leukemias (marked by an increase in circulating lymphocytes) are rare in horses, but lymphomas (solid tumors of lymphocyte origin) are relatively common, especially in older horses. The cause of the tumors is unknown, but a viral etiology is suspected. We have identified leukemias of both the B and T lymphocyte populations; to our knowledge this is the first such characterization of horse leukemias. A case of immunodeficiency sent to us from the University of Pennsylvania was characterized by an absence of B lymphocytes and a deficiency in MHC antigen expression on the animal's T lymphocytes. The affected horse experienced repeated infections that eventually resulted in its death. Although the information we have obtained is not yet useful in a clinical sense, studies of this sort are necessary if clinical veterinary medicine is to continue to advance. In the meantime, our research has provided valuable material for studies of the immune system of the horse.

*Douglas F. Antczak*

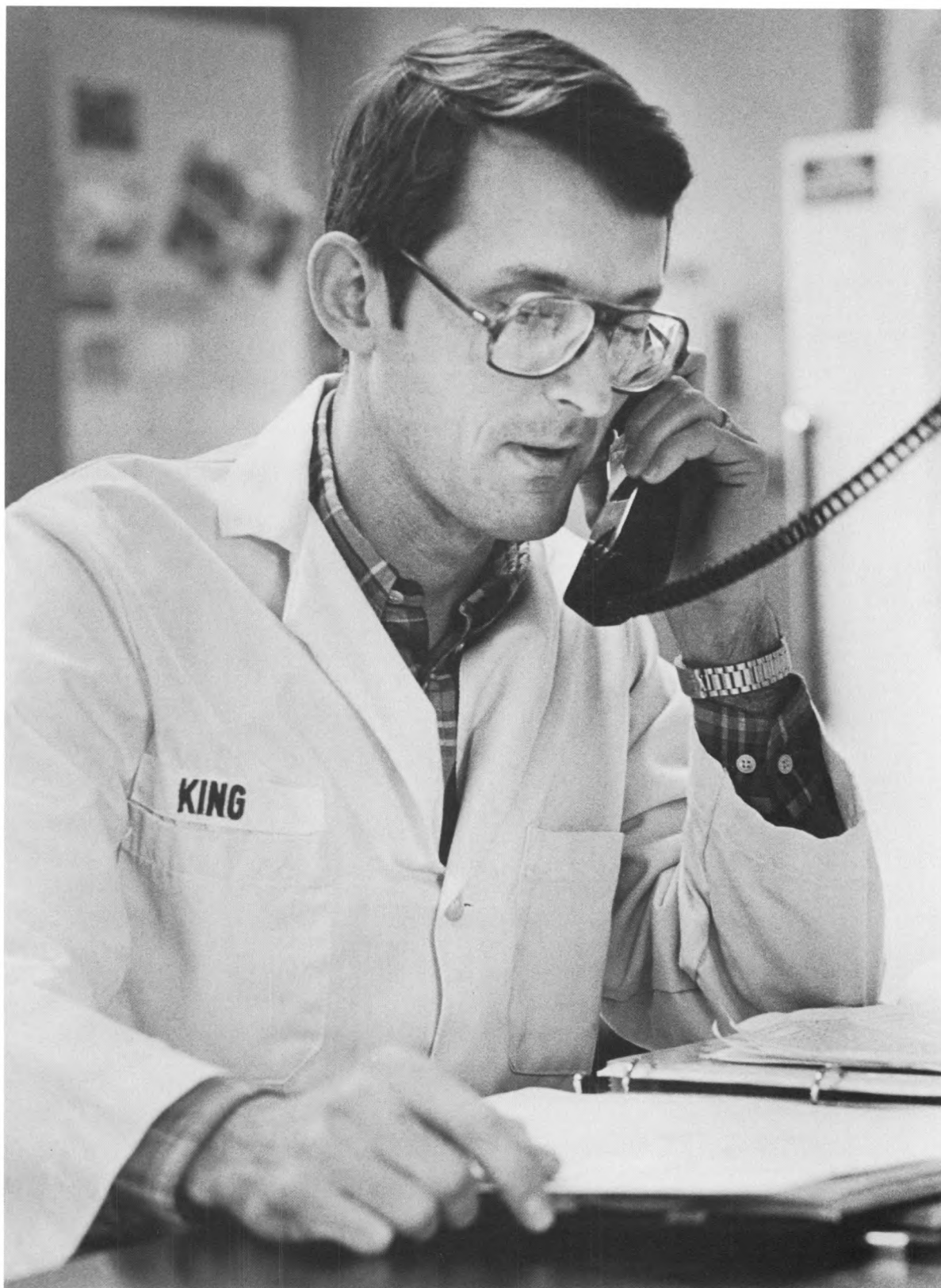


Cheryl Rigas



Robert Wakeman

*We have been  
studying how  
laboratory rats cope  
with a Listeria  
monocytogenes  
infection.*



Alan King

## Donnelley Laboratory of Immunochemistry

The ability of cells to internalize and kill bacteria is a primitive defense against infection. That ability operates efficiently against a wide variety of microorganisms; however, some bacteria have evolved the capacity to resist intracellular activation. Such organisms are known for their capacity to cause serious and often chronic infections in both animals and humans. They include the causal agents of tuberculosis, brucellosis, listeriosis, and Legionnaire's disease, to name a few.

Alan King, a veterinarian on leave from the United States Army, has been studying how laboratory rats cope with a *Listeria monocytogenes* infection. Last year we reported that infected animals generate a population of killer lymphocytes that can protect recipient rats against an *L. monocytogenes* challenge. The protective cells were tentatively identified as thymus-dependent (T) lymphocytes rather than as another category of lymphocytes called natural-killer (NK) cells. This year Dr. King developed further evidence that T cells are indeed the mediators of this defense. Using the fluorescence-activated cell sorter, he and Tom Bartol successfully separated *L. monocytogenes*-protective cells into a fraction that comprised only a tiny portion of the animal's total lymphocyte population. The cells of interest had a surface antigenic profile characteristic of cytotoxic T cells. Dr. King also demonstrated that the cells were rich in lysosomes, a finding that encourages the belief that the protective cells have a cytotoxic capacity. Dr. King was awarded a Ph.D. degree in December 1989 for his work on experimental listeriosis. He has now been reassigned to the army's research laboratory in Bangkok, Thailand.

*Douglas D. McGregor*



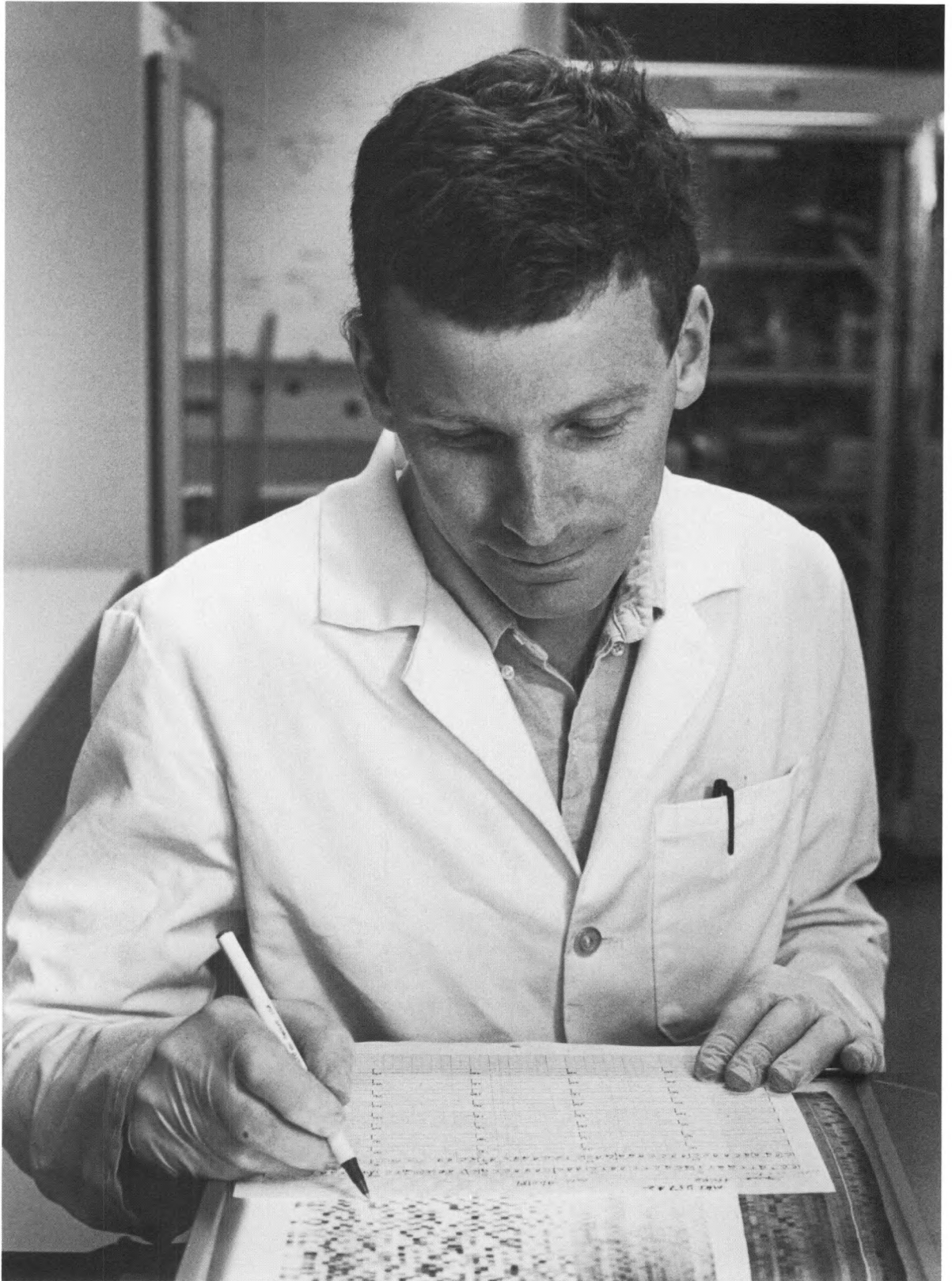
Mark Hulsebosch



Anita Hesser



*We have been part  
of a continuing  
effort at the Baker  
Institute to apply  
new methods of  
molecular biology  
to the study of  
infectious  
diseases.*



Colin Parrish



## Bostwick Laboratory of Molecular Biology

Animal viruses have the capacity to infect and cause disease in many species. Although the host ranges of many viruses are sharply circumscribed, it is possible in rare instances for a virus to change in ways that enable it to infect animals of a previously resistant species. Since prehistory such evolutionary changes have given rise to the present patterns of host susceptibility to viral diseases. A host-range alteration probably accounts for the sudden emergence of canine parvovirus (CPV-2) in the late 1970s as a serious and previously unrecognized disease of dogs.

Much of the research in this laboratory is directed toward the question of how CPV-2 acquired the capacity to infect dogs. We are confident that the virus first appeared during the 1970s, but we do not know for certain where or from what ancestral virus it evolved. Feline panleukopenia virus (FPV), or a closely related parvovirus of a wildlife species such as raccoons or mink, is a likely candidate. All of those parvoviruses have remarkably similar genetic structures, so even a minor change in the genome of the ancestral virus from which CPV derived might have given it the capacity to infect and grow in dogs.

Our research findings support this notion. Through the use of the genetic techniques of DNA cloning and sequencing and through analyses with highly specific monoclonal antibodies prepared against CPV and FPV, we have shown that only a small number of differences in the DNA sequences of the subject viruses determine their host ranges. The genetic differences governing the host restrictions of CPV and FPV have been mapped to a region that codes for a part of the virus protein coat. Since this critical region represents a very small portion of the viral genome, it follows that even a few mutations in this region of the FPV genome could account for a dramatic change in its behavior, including its ability to replicate in dog cells.

Our efforts at the moment focus on more closely defining the area of the genome that codes for the capsid gene of FPV. For example,

we have found that the changes in the genome that enable variant viruses to infect dog cells take away their capacity to replicate in cat cells. Presumably, this is another feature of the evolutionary process that accounts for the individual, highly restricted host ranges of these viruses.

On another front, we are collaborating with researchers at Purdue University who are using X-ray crystallography to determine the three-dimensional structure of viruses. Our objective is to determine how a natural or experimentally induced mutation in the capsid gene influences the shape of the virus. Aside from its fundamental interest for molecular virologists, an understanding of how the structure of a virus affects its capacity to infect cells may provide insights into ways of impeding the process.

During 1979 and 1980 we isolated a variant of CPV-2 from the dog population. This new strain, CPV-2a, has become globally distributed and has largely replaced the original CPV-2 virus in most dog populations. The difference between CPV-2 and CPV-2a may again be related to a property of the capsid protein, and we intend to test this hypothesis by identifying and comparing nucleic acid sequences of the capsid protein genes of the subject viruses. We will also examine other properties of CPV-2a that might account for its selective advantage over CPV-2.

In the course of our studies we have taken part in a continuing effort at the Baker Institute to apply new methods of molecular biology to the study of infectious diseases. By developing those methods and acquiring the highly specialized equipment required for those purposes, we are creating a core facility that will make molecular techniques available to all Institute laboratories, thereby facilitating ongoing research, fostering collaboration, and maintaining our preeminence in infectious disease research.

*Colin R. Parrish*

## Publications

The following is a list of manuscripts published by staff members of the Baker Institute in 1989. Publications listed as in press in last year's report are repeated this year, with their original numbers, to record their full bibliographic details.

- 732 **Chunekamrai, S., L. P. Krook, G. Lust, and G. E. Maylin.** 1989. Changes in articular cartilage after injections of methyl-prednisalone acetate into equine joints. *Am. J. Vet. Res.* 50:1733-41.
- 738 **Steinetz, B. G., L. T. Goldsmith, H. J. Harvey, and G. Lust.** 1989. Plasma relaxin and progesterone in pregnant, pseudopregnant, and ovariectomized, progestin-treated pregnant dogs: Relaxin as a marker for pregnancy. *Am. J. Vet. Res.* 50:68-71.
- 746 **Allen, W. R., J. Kydd, and D. F. Antczak.** In press. Xenogeneic donkey-in-horse pregnancy created by embryo transfer: Immunological aspects of a model of early abortion. In *The immunology of the fetus*, ed. G. Chaouat. Boca Raton, FL: CRC Press.
- 758 **Burton-Wurster, N., and G. Lust.** In press. Fibronectin in cartilage. In *Fibronectin in health and disease*, ed. S. Carsons. Boca Raton, FL: CRC Press.
- 759 **Burton-Wurster, N., and G. Lust.** 1989. Molecular and immunologic differences in fibronectin from articular cartilage and plasma. *Arch. Biochem. & Biophys.* 269:32-45.
- 760 **Carlisle, M. S., D. D. McGregor, H. O. Mohammed, and J. A. Appleton.** 1989. The kinetics of antibody mediated mucus entrapment and rapid expulsion of *Trichinella spiralis* muscle larvae. In *Proceedings of the seventh international conference on trichinellosis*, ed. C. E. Tanner, 141. Madrid: Consejo Superior de Investigaciones Cientificas Press.
- 761 **Carmichael, L. E.** In press. Canine brucellosis. In *Animal brucellosis*, ed. K. Nielsen and J. R. Duncan. Boca Raton, FL: CRC Press.
- 762 **Carmichael, L. E., J. C. Joubert, and L. Jones.** 1989. Characterization of *Brucella canis* protein antigens of polypeptide antibody responses of infected dogs. *Vet. Microbiol.* 19:373-87.
- 765 **Korenaga, M., C. H. Wang, R. G. Bell, D. Zhu, and A. Ahmad.** 1989. Intestinal immunity to *Trichinella spiralis* is a property of OX-8<sup>+</sup> OX-22<sup>+</sup> T-helper cells that are generated in the intestine. *Immunology* 66:588-94.
- 767 **Leipold, H. R., R. L. Goldberg, and G. Lust.** 1989. Canine serum hyaluronate and keratan sulfate concentrations: Relationship to age and osteoarthritis. *Arth. and Rheumat.* 32:312-21.
- 772 **Zhu, D., and R. G. Bell.** 1989. IL-2 production, IL-2 receptor expression and IL-2 responsiveness of spleen and MLN cells from inbred mice infected with *Trichinella spiralis*. *J. Immunol.* 142:3262-67.
- 773 **Ahmad, A., C. H. Wang, M. Korenaga, R. G. Bell, and L. S. Adams.** In press. Synergistic interaction between immune serum and thoracic duct cells in the adoptive transfer of rapid expulsion of *Trichinella spiralis* in adult rats. *Exp. Parasitol.*

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- 774 **Allen, W. R., D. F. Antczak, and J. F. Wade**, eds. 1989. Equine embryo transfer II. *Equine Veterinary Journal Supplement* 8:1-133.
- 775 **Antczak, D. F.** 1989. Maternal antibody responses in pregnancy. *Current Opinion in Immunology* 1:1135-40.
- 776 **Antczak, D. F., and W. R. Allen.** 1989. Maternal anti-fetal immune responses in xenogeneic pregnancy. In *Xenograph* 25, ed. M. A. Hardy, 221-35. Amsterdam: Elsevier Science Publishers.
- 777 **Appleton, J. A., L. F. Gagliardo, D. F. Antczak, and C. Poleman.** In press. Production of an equine monoclonal antibody specific for the H7 hemagglutinin of equine influenza virus. *Vet. Immunol. Immunopathol.*
- 778 **Blakemore, W. F., B. A. Summers, and M. J. G. Appel.** 1989. Evidence of oligodendrocyte infection and degeneration in canine distemper encephalomyelitis. *Acta Neuropathol.* 77:550-53.
- 779 **Bull, R. W., H. A. Lewin, M. C. Wu, K. Peterbaugh, D. F. Antczak, et al.** 1989. Joint report of the third international bovine lymphocyte antigen (BoLA) workshop, 27 July 1986, Helsinki, Finland. *Animal Genetics* 20:109-32.
- 780 **Burton-Wurster, N., G. Lust, and R. Wert.** 1989. Expression of the ED-B fibronectin isoform in adult human articular cartilage. *Biochem. Biophys. Res. Comm.* 165:782-87.
- 781 **Carlisle, M. S., D. D. McGregor, and J. A. Appleton.** In press. The role of mucus in antibody mediated rapid expulsion of *Trichinella spiralis* in suckling rats. *Immunology.*
- 782 **Carmichael, L. E.** 1989. Canine parvovirus immunization: Myths and realities. *A. K. C. Gazette*, December, 94-102.
- 783 **Oriol, J. G., J. C. Poleman, D. F. Antczak, and W. R. Allen.** 1989. A monoclonal antibody specific for equine trophoblast. *Eq. Vet. J. Suppl.* 8:14-18.
- 784 **Parrish, C. R.** In press. The emergence, natural history and variation of canine, mink and feline parvoviruses. *Advances in Virus Research.*
- 785 **Pearce-Kelling, S., W. J. Mitchell, B. A. Summers, and M. J. G. Appel.** In press. Growth of canine distemper virus in cultured astrocytes: Relationship to *in vivo* persistence and disease. *Microbial. Pathogenesis.*
- 786 **Zhu, D., and R. G. Bell.** In press. Antigen-specific lymphocyte proliferative responses in inbred mice after *Trichinella spiralis* infection. *J. Parasitol.*
- 787 **Zhu, D., and R. G. Bell.** In press. *Trichinella spiralis*: Murine strain variation in response to monoclonally defined, protective, non stage-specific antigens. *Exp. Parasitol.*



*Those who support  
the work of the  
Institute know that  
it takes a sense  
of mission—a  
commitment not just  
to excellence, but also  
to the greater good.*



*Photograph by C. H. Brown*

## The Institute in Perspective

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Our report looks back on another year of rewarding progress in Baker Institute research. Some immediate benefits are clear, as in Skip Carmichael's development of more-sensitive methods of screening for canine brucellosis, or in Nancy Burton-Wurster's replication in tissue cultures of changes in cartilage metabolism that, until now, have been observed only in animal subjects. Naturally, some anticipated benefits will take longer to accrue. Although we direct our efforts to the health needs of dogs and horses, the answers we seek are fundamental to questions of infection and immunity that apply to many species. Thus, some findings that come from research intended for the benefit of dogs will deepen our understanding of diseases in general and our own health and well-being in particular.

The research described in this report makes use of sophisticated technology, and few of us fully understand its subtleties or implications. It may be difficult to relate such activities as mapping genomes or cleaving antibodies to our own experience, and therefore difficult to place them in a broader human context. Although we all know that people create scientific breakthroughs, and though we can enumerate many of the requisite attributes of a good scientist, not everyone recognizes that it takes more than a fortuitous combining of talent, curiosity, training, and effort to build success on success as the men and women of the Baker Institute have done for decades. It takes a sense of mission—a commitment not just to excellence, but also to the greater good. Those who support the work of the Institute know that and share our sense of mission. As the following pages attest, their numbers are strong, and growing. Therefore we proudly dedicate our report to the many individuals who have so generously demonstrated their own commitment to bettering the lives of animals—for all our sakes.

*William Rockefeller*  
*Chairman*

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*We were deeply saddened to learn at press time of the death of Bill Rockefeller on March 16, 1990. Bill lent his guidance and support to many worthy efforts during his lifetime, but perhaps none was more important to him than the cause of animal welfare. We shall remember him with gratitude and affection.*

*D. D. McG.*

*A toast to our friend  
Lottie Yates, who  
celebrated her one  
hundredth birthday  
with her companion,  
French, at her side.*





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 memory of Sugar)  
 Mr. and Mrs. Arthur Christiansen  
 (in memory of Alfie)  
 Mr. and Mrs. Robert Christie (in  
 memory of Velvet)  
 Dr. Nicholas Chuff  
 Dr. Michael T. Chumrau  
 Mrs. Everett M. Clark (in memory  
 of Merry Lee's Mr. Woo of  
 Mirolac)  
 Mrs. Frances P. Clark (in memory  
 of Dumpsty)  
 Mr. and Mrs. Gerald W. Clark  
 (in memory of Mitzie)  
 Ms. Laura Clark  
 Mrs. Marcelle S. Clark (in  
 memory of Buffy and Beau)  
 Dr. Peter Clary  
 Mr. Howard Clements  
 Ms. Linore Cleveland  
 Ms. Gwendoline R. Cline  
 Dr. Charles Clinton  
 Dr. Angela Sue Clover  
 Mr. and Mrs. Donald Coager (in  
 memory of Heidi Ann)  
 Mr. and Mrs. Rodney L. Cockrell  
 Dr. Susan Coggins  
 Dr. and Mrs. Alan D. Cohen (in  
 memory of Trophy and in  
 honor of Wild)  
 Dr. Jerrold Cohen  
 Mr. and Mrs. John Cole  
 Dr. Susan H. Cole  
 Mr. and Mrs. Leonard A. Colella  
 (in memory of Phebe Conover)  
 Mr. and Mrs. Robert L. Coleman  
 Ms. Lillian E. Coletti (in memory  
 of Abel and Cain)  
 Dr. David S. Coley  
 Mr. and Mrs. Harold C. Colley  
 (in memory of Dudley)  
 Dr. Linda L. Collier  
 Ms. Beverly Colman (in memory  
 of Ashley)  
 Ms. Rosabel Colman (in memory  
 of Ashley)  
 Dr. Carolyn F. Comans  
 Dr. Edmond A. Comans  
 Mr. and Mrs. Charles R.  
 Comerkeski

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Companion Animal Hospital (New York)	Mr. and Mrs. Willard R. Daggett (in memory of Phebe Conover)	Mrs. Donna I. Dempster-McClain
Companion Animal Hospital (Virginia)	Mrs. Stephanie Dalbey	Ms. Mabel Q. Dennis (in memory of Shep)
Ms. Carolyn Ann Cone (in memory of Princess)	Mr. and Mrs. Joseph J. Daley (in memory of Radar, Mickey, Jessica, Jamie, Magic, Sam, and in memory of Mr. Angelo [Eddie] Billelo)	Mr. and Mrs. Richard J. DeRuyscher (in memory of Goldie)
Mr. and Mrs. Carl P. Conte	Mrs. Carol Daniels (in memory of Gillie)	Ms. Jane Desher (in memory of Sam)
Ms. Ann M. Conway (in memory of Chubba)	Dr. Margaret L. Darcy	Ms. Elizabeth DeVito (in memory of Sander)
Miss Mary Lee Cooke	Dr. Douglas Darlington	Mr. and Mrs. Michael J. DeVito
Mrs. Marcelle Cooney	Dr. Craig Datz	Dr. Marjorie A. Dewey
Dr. Frank H. Coons	Mr. and Mrs. Wilbur F. Daub (in memory of Tiger)	Ms. Dorothy E. DeYoung (in memory of Butch)
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Miss Marion Foetsch (in memory of Hominy)  
Mrs. Jean H. Fontaine (in memory of Scott Fisher)  
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Mr. and Mrs. Joseph Fosorile (in memory of Sheba)  
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Dr. Raymond M. Fournier  
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Dr. David Fox  
Ms. Mary Francis (in memory of Rip)  
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Dr. and Mrs. Richard O. Gale (in memory of Buffy)	Dr. Joseph Glennon	Mr. Kenneth W. Gray (in memory of Tam)
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Dr. Nola Z. Gedeon	Mr. and Mrs. Harvey M. Goldstein (in honor of Thomas Lonergan)	Mr. and Mrs. Milton D. Grosse
Mr. and Mrs. Hillel Gedrich (in memory of Ralph and in honor of Dr. Brian Green)	Dr. Norman H. Goldstein	Mrs. Judith Grotsky (in memory of Cotton)
Ms. Genise M. George	Ms. Rosalind Goltz (in memory of Leon and Wooly)	Ms. Gertrude M. Grunenwald
Mr. and Mrs. John George	Mr. and Mrs. Alvin Goodman (in memory of Phebe Conover)	Dr. Edward J. Gschrey
Dr. Lisa Geovjian	Mr. and Mrs. Edward A. Goodman, Jr. (in memory of Mr. Art Ross)	Dr. Fred A. Guenther
Dr. William A. Gerber	Dr. Gerald R. Goodman	Dr. Joanna Guglielmino
Mr. Erwin Gerboth	E. Jane Goplerud, M.D. (in memory of Brandy)	Dr. Louis Guida
Mrs. Frances M. Gerdes	Dr. Edward I. Gordon	Dr. Marguerite Gulick
Mrs. Ruth H. Geringer (in memory of Link)	Mrs. Melanie R. Gordon	Mr. Anthony C. Gulino (in memory of Rachel)
Dr. Eric L. Germaine	Mr. Peter L. Gordon	Dr. Fern Gurock
German Flatts Veterinary Clinic	Dr. Ferris G. Gorra	Dr. Harlan C. Gustafson
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Ms. Alice K. Gilbert (in memory of Yofie)		Dr. Richard Hahn
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		Mr. and Mrs. Sheldon Handschuh
		Mrs. Eleanor Hanna (in memory of Brandy and Quinn)
		Dr. Patricia Hannegan
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Dr. Michael Hardaker	Ms. JoAnne Herrera	Dr. Priscilla S. Irwin
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Dr. Donald W. Hartrick*	Mrs. Clinton E. Hodgins	Dr. Karen Johnson
Mrs. Judith E. Harvell (in honor of Chief and Choo Choo)	Mr. and Mrs. John G. Hoffman (in memory of LouLou)	Dr. Lisa W. Johnson
Mr. Stewart G. Hastings (in memory of Mitzie)	Dr. Frederick M. Holmes	Dr. Tom J. Johnson
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Mr. William Hawkinson (in memory of Connie Beckhardt)	Ms. JoAnn Holmes	Dr. Herbert Jonas
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Mr. and Mrs. Norman H. Heaven	Dr. Courtland R. Howard	Dr. Abe B. Kamine
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Dr. Kathleen Hefner	Dr. Vikki Howard	Mr. and Mrs. Steven Kanterman
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Dr. Joseph Helfand	Mr. and Mrs. Manly R. Hubbs	Mrs. Barbara D. Kaspar
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 Dr. Kathleen King  
 Mr. and Mrs. William A. King (in memory of Scott Fisher)  
 Mr. and Mrs. Charles R. Kingsbury  
 Mr. and Mrs. Kingdon E. Kingsley (in memory of Rusty and Shasta)  
 Mr. and Mrs. Jack J. Kirchner  
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 Mr. and Mrs. Paul C. Tracy (in  
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 Miss Susan Frances Train  
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 Village Veterinary Hospital  
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and Midnight)  
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Dr. Ernest Willers  
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Dr. Russell D. Williamson  
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Jackson)  
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Albany Obedience Club (in memory of Phebe Conover)  
 Allentown Dog Training Club\*  
 American Boxer Club†§  
 American Maltese Association\*  
 Anne Arundel Kennel Club‡  
 Back Mountain Kennel Club‡  
 Cairn Terrier Club of America\*  
 Chesapeake Kennel Club\*  
 Columbiana County Kennel Club†  
 Corpus Christi Kennel Club (in memory of Mozelle Lambert)\*  
 Delaware Valley Afghan Hound Club  
 Devon Dog Show Association‡  
 Dog Owners Training Club of Maryland  
 Elm City Kennel Club\*  
 Evergreen Empire Manchester Terrier Fanciers  
 Farmington Valley Kennel Club†  
 Finger Lakes Kennel Club†§  
 German Shepherd Dog Club of Western New York (in honor of Dr. John C. Laurie)  
 Goldsboro Kennel Club  
 Greater Lowell Kennel Club†  
 Harrisburg Kennel Club‡  
 Huntsville Obedience Training Club\*

International Kennel Club of Chicago‡  
 Ithaca Dog Training Club\*§  
 Kanadasaga Kennel Club†§  
 Kennel Club of Buffalo (in honor of Dr. John C. Laurie)\*§  
 Labrador Retriever Club of Central Connecticut (in honor of Guilford Veterinary Hospital)  
 Los Alamos Dog Obedience Club  
 Meadowbrook Cocker Spaniel Club  
 Medina Kennel Club (in memory of Mrs. Harrison Fagan)\*  
 Middle Atlantic States Komondor Club\*  
 Minnesota Field Trial Association  
 Monticello New York Kennel Club (in honor of Doctors Bartholf, Cone, Hahn, Kross, Mauer, Nebzydoski, Stein, Tintle, and Wachter)\*  
 National Capital Kennel Club (in memory of H. Richard and Virginia W. Garrett)†  
 Newfoundland Club of America\*  
 Newtown Kennel Club‡  
 Niagara Frontier Obedience Association\*  
 North Shore Dog Training Club  
 Northwestern Connecticut Dog Club (Muriel and Herbert Kaye Memorial Fund for Canine Research and in honor of Dr. Paul J. Cortesi)\*

Norwich and Norfolk Terrier Club\*  
 Old Pueblo Dog Training Club\*  
 Olympic Kennel Club\*  
 Ox Ridge Kennel Club‡  
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 Rockland County Kennel Club‡  
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 Saratoga Kennel Club (in honor of Dr. Robert Sofarelli)  
 Saw Mill River Kennel Club†§  
 Somerset County Dog Obedience Club\*  
 Spartanburg Kennel Club (Betsy Holland Memorial Fund)†  
 Springfield Kennel Club†  
 Suburban Dog Training Club  
 Tioga County Club  
 Troy Kennel Club\*  
 Two Cities Kennel Club\*  
 Ulster Dog Training Club\*  
 Valley Hills Obedience Club\*  
 Wolverine Dog Training Club (in memory of Heidi)  
 Yosemite Kennel Club†

\*Gave \$100-\$499 since January 1, 1989.

†Gave \$500-\$999 since January 1, 1989.

‡Gave \$1,000 or more since January 1, 1989.

§Institute founder.

## Associations

American Kennel Club†§

Women's Auxiliary to the Long Island Veterinary Medical Association

Women's Auxiliary to the New York State Veterinary Medical Association

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 Animal Inn\*  
 AT&T  
 Atlantic Monthly Company (in memory of Scott Fisher)\*

Ciba-Geigy Corporation‡  
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Mr. Angelo (Eddie) Billelo  
Mr. William Campos  
Mr. Anthony Christina  
Mr. Harvey Cohen  
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*There are many ways  
you can give to  
advance our work.*



*Photograph by C. H. Brown*

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James A. Baker Institute for Animal Health  
Cornell University  
Ithaca, New York 14853

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